

Assessment of the Potential Risk to Workers from Exposure to 1,3-Butadiene

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The available epidemiologic data provide equivocal evidence that 1,3-butadiene is carcinogenic in humans; some available studies suggest that the lymphopoietic system is a target, but there are inconsistencies among studies in the types of tumors associated with 1,3-butadiene exposure, and there is no evidence of a relationship between length of exposure and cancer risk, as one might expect if there was a true causal relationship between 1,3-butadiene exposure and cancer risk. The available chronic animal studies, however, show an increase in tumor incidence associated with exposure to high concentrations of 1,3-butadiene. In addition to the general uncertainty of the relevance of animal data to humans, there are several additional reasons why the National Toxicology Program's mouse study may not be appropriate for assessing possible human risks. These include: *a*) the possible involvement of a species-specific tumor virus (MuLV) in the response in mice; *b*) apparent differences between mice and humans in the rate of metabolism of 1,3-butadiene to reactive epoxides that may be proximate carcinogens; *c*) use of high dose levels that caused excess early mortality; and *d*) exposure of animals to 1,3-butadiene for only about half their lifetime. While recognizing the uncertainty in using the available animal data for risk assessment, we have performed low-dose extrapolation of the data to examine the implications of the data if humans were as sensitive as rats or mice to 1,3-butadiene, and to examine how the predictions of the animal data compare to that observed in the epidemiologic studies.

With the mouse data, because the study was of less than lifetime duration, we have used the Hartley-Sielken time-to-tumor model to permit estimation of lifetime risk from the less than lifetime exposure of the study. With the rat data, we have used three plausible models for assessing low-dose risk: the multistage model, the Weibull model, and the Mantel-Bryan probit model. With both the rat and mouse data, we used information on how much 1,3-butadiene is retained by animals exposed to various concentrations of the chemical. This improves the accuracy of the low-dose extrapolation. When extrapolated to low-dose levels, mice appear to be at greater risk (by a factor of 5-fold to 40-fold) than rats. Some of this difference (a factor 3-fold to 5-fold) may be due to the faster rate of metabolism of 1,3-butadiene to, and higher blood levels of, epoxide derivatives in mice than in rats.

If humans, rats, and mice were at equal risk from equal average lifetime daily doses of retained 1,3-butadiene, the mouse and rat data would predict risks to occupationally exposed humans that are statistically inconsistent at the 95% level with the results of the available human data, unless human exposure in the past was very much lower than is believed to have been the case.

Introduction

The risk assessment of 1,3-butadiene described here was conducted in 1986. The purpose of the assessment was to perform an independent quantitative risk assessment of 1,3-butadiene, with full consideration and appropriate evaluation of all data available at that time, as an alternative to the assessments conducted by the U.S. Environmental Protection Agency (EPA) (1,2). Careful attention was paid to the important components of a risk assessment that were identified by the National Re-

search Council (3). These are *a*) hazard identification, which is the determination of whether or not a particular chemical is causally linked to particular health effects; *b*) dose-response evaluation, which is the determination of the relation between the magnitude of exposure and the probability of occurrence of the health effects in question; *c*) exposure assessment, which is the determination of the extent of human exposure that is anticipated before or after application of controls; and *d*) risk characterization, which is the description of the nature and magnitude of human risk, including attendant uncertainty.

Each of these stages is associated with some degree of uncertainty. A comprehensive risk assessment attempts to reduce the uncertainties at each step as much as possible. At the same time, remaining uncertainties

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must be clarified and described so that an accurate picture is presented of the relative uncertainties in estimating the risk of any particular chemical.

Most of the analysis presented here is based on data available in 1986. Where new data have become available that might alter this analysis, these are mentioned, but we have not recalculated any of the risk estimates based on the new data.

Hazard Identification

The purpose of hazard identification is the qualitative determination of the range of toxic effects that a substance is capable of causing and the conditions of exposure under which those effects occur. During this stage of risk assessment, all experimental data pertaining to the toxicity of the test substance are comprehensively and critically evaluated to assess their reliability in demonstrating causation between level of exposure to the test substance and the health effect identified. Those studies demonstrating the most reliable evidence of causation will be those considered for use in the next step of risk assessment, dose-response evaluation.

In the current case, the hazard of interest is the potential to cause cancer. The three major types of data considered in carcinogenic hazard identification are from studies in humans (clinical and epidemiologic data), long-term experimental animal studies, and other biological systems, particularly genotoxicity assays (assays for mutations and other genetic changes).

Epidemiology Studies

The EPA (2) reviewed a number of epidemiological studies of occupational cohorts with potential exposure to 1,3-butadiene. Several of these studies involved facilities that produced natural rubber products in addition to synthetic styrene-butadiene rubber (4-8). The interpretation of such studies, with respect to the potential association of 1,3-butadiene and health effects, however, is limited as a result of the likely confounding effects of cohort exposure to a number of other potential carcinogens in nonbutadiene operations. Furthermore, it is unlikely that 1,3-butadiene was present in significant quantities in these facilities. The two remaining studies evaluated by EPA (2), however, involved workers at styrene-butadiene manufacturing plants whose primary exposure was 1,3-butadiene (9,10). Since the publication of the EPA assessment, an additional epidemiologic study of a cohort of 1,3-butadiene production workers has been reported (11). In the following, we will limit our analysis to the latter three studies that involved workers primarily exposed to 1,3-butadiene and in whom other exposures were less likely to confound a potential 1,3-butadiene-cancer association.

Review of the three relevant epidemiologic studies of 1,3-butadiene-exposed workers indicates that in all of the studies, mortality from all causes of death combined and from all malignant neoplasms combined was less

than would be expected, based upon national mortality data. When one examines the results for specific cancer sites, only one significant elevation, for lymphosarcoma and reticulosarcoma combined, in one cohort was observed when expected mortality was based upon national mortality data, but not when it was based upon local data (12). When the cohort was broken down by work area, a significant excess was observed only in the routine exposure group. The lymphosarcoma and reticulosarcoma cases were concentrated in short-term workers in this exposure group, however, arguing against a causal association.

Certain cancer sites or groupings in these studies revealed increases above expectation which, although not statistically significantly elevated, deserve further attention.

In the Matanoski et al. (10,12) study, standardized mortality ratios (SMRs) in white males that approached or slightly exceeded expectation were those for cancers of the digestive organs (SMR = 98), larynx (SMR = 109), kidney (SMR = 103), and Hodgkin's disease (SMR = 128); SMRs for all causes and for all cancers were 78 and 83, respectively. Workers in the production area, who may have involved relatively higher levels of 1,3-butadiene exposure, showed few elevated mortality ratios. SMRs for the previously listed sites of concern were generally lower than those for workers in the other work areas that were considered. This argues against 1,3-butadiene being a causal factor in these elevated SMRs. When these sites of concern from the Matanoski study were compared with the study results of Meinhardt et al. (9) and Downs et al. (11), no consistent elevations were observed.

In one of the two plants studied by Meinhardt et al. (9) nonstatistically significant increases (based on more than one case) in cancers were observed in the lymphatic and hematopoietic tissues combined; also lymphosarcoma, reticulosarcoma, leukemia, and aleukemia were observed. The interpretation of the meaning of increases in the grouping of lymphatic and hematopoietic cancers is difficult because of limitations in scientific knowledge about their individual etiologies. It is generally accepted, however, that the leukemias encompass a diverse group of malignancies with difference cell origins: pathogenesis, age, race and sex distributions, indicated therapies, and likely etiologies (13,14). Similarly, the various malignant lymphomas are considered to represent different biological entities, with Hodgkin's disease representing a unique pathologic process and the remaining (non-Hodgkin's) lymphomas falling into two major groups, each of which is further divided into a number of cytologic subcategories (15,16). Although epidemiologists have often lumped the lymphohematopoietic tumors together, the distribution of such diseases and their clinical and pathologic diversity suggest they likely represent different etiologic entities. It is therefore more appropriate to consider each lymphohematopoietic malignancy separately when analyzing epidemiologic data.

The increase in lymphosarcoma and reticulosarcoma in this plant studied by Meinhardt et al. (9) should be interpreted in light of the other available study results. Although an increase in this diagnostic grouping was observed in the Texaco plant studied by Downs et al. (11), the concentration of cases in short-term workers in the routine exposure group suggests 1,3-butadiene was not the likely causal factor. The SMR for this diagnostic group was significantly decreased in the larger Matanoski study, and only slightly increased in the other plant studied by Meinhardt et al. (not statistically significant, based on one case). Overall, the absence of an increase in the larger Matanoski study, the fact that increases were generally based upon small numbers of cases, and that increases were not reported to apply specifically to lymphosarcoma or reticulum cell sarcoma tends to argue against a causal association.

Upon closer examination, a causal association between 1,3-butadiene and the leukemias in plant A of the Meinhardt study was not supported. This is because the latency period was extremely short (less than 3 years) in two cases, and in the remaining cases with sufficient latency, leukemia cell types were diverse. A similar excess of leukemia was not seen in the other SBR plant studied by Meinhardt, nor in Matanoski et al. (10,12). A slight excess of leukemia was observed in the Downs et al. study (11), but as it was based upon only one excess death over expectation, its significance is unclear.

Although each of these studies made some attempt to categorize workers as to past 1,3-butadiene exposure, the lack of past industrial hygiene monitoring data makes interpretation of such categorized data extremely difficult. Furthermore, in each study analysis of such crude exposure, class data generally failed to show consistent patterns that would suggest dose-response associations. This general lack of data suggestive of a dose-responsive gradient for end points of concern further limits conclusions regarding potential causality.

These three critical epidemiologic studies have been updated, and new information was presented at the International Symposium on the Toxicology, Carcinogenesis, and Human Health Aspects of 1,3-Butadiene held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. This new information does little to clarify the role of 1,3-butadiene in carcinogenesis in humans.

Lemen et al. (17) present some preliminary information from continued follow-up of the cohort studied by Meinhardt et al. (9). They report greater incidences of lymphosarcoma and reticulosarcoma and cancers of the trachea, bronchus, and lung; they do not state whether these increases in numbers were associated with increases in SMRs. It will be important to evaluate this updated information when the full data are available.

Divine (18) reported some updated information on the Texaco study (11). The overall pattern of results was not changed in the update, with an overall reduction in expected mortality (SMR = 84), but a significantly elevated SMR for lymphosarcoma and reticulosarcoma

(SMR = 229). This increase was associated with individuals employed less than 10 years and first hired during World War II, however. The absence of an association between length of exposure and cancer risk raises questions regarding the causal role of 1,3-butadiene exposure in the excess.

Matanoski (19) presented an update of her studies of workers in eight styrene-butadiene rubber manufacturing facilities (10,12). As in her earlier reports, Matanoski reported reduced overall mortality (SMR = 81) among the workers. In this update, however, she reported a significant excess of leukemia among black production workers (SMR = 656) but not white workers (SMR = 84), and of non-Hodgkin lymphoma in production workers (combined races, SMR = 260). In contrast to the studies of Meinhardt et al. and Downs et al. (9,11,17,18), no deaths increased because of lymphosarcoma. Although this new finding does raise some concern about the lymphopoietic system as a target in humans exposed to 1,3-butadiene, the lack of a consistent pattern of tumor type calls into question the causative role of 1,3-butadiene in the reported tumor excess.

In conclusion, the epidemiologic data show that occupational exposure to 1,3-butadiene is not associated with an excess risk of mortality from all causes or from all cancers combined. There is some evidence of an excess of lymphopoietic neoplasms in several studies, but the increases were in different tumor types in different studies (lymphosarcoma in some studies, leukemia in others) and no clear evidence was found for an association between duration of employment and tumor rate. The absence of a consistent pattern of affected tumor types within and across studies and the absence of past industrial hygiene data make any conclusions about causality tentative. Overall, however, the available epidemiologic data do not provide strong evidence of a causative relationship between human exposure to 1,3-butadiene and elevated cancer mortality of any type.

NTP Mouse Bioassay

In a National Toxicology Program (NTP) sponsored chronic bioassay, groups of 50 male and female B6C3F₁ mice were exposed to air containing 0 (chamber controls), 625, or 1250 ppm 1,3-butadiene for 6 hr/day, 5 days/week (20). Exposures were planned to continue for 103 weeks, but they were terminated at week 60 for male mice and week 61 for female mice primarily because of neoplasia contributing to rapidly declining survival.

Significantly increased numbers of neoplasms were observed at multiple sites in both sexes at both low and high doses. Incidences of the most prevalent neoplasms are summarized in Table 1. In addition to the lymphomas, cardiac hemangiosarcomas, and alveolar/bronchiolar adenomas/carcinomas listed in Table 1, there was an increased incidence of epithelial hyperplasia, papillomas, and squamous cell carcinomas in the forestomach in 1,3-butadiene-exposed mice of both sexes. Among females, acinar cell carcinomas of the

Table 1. Summary of incidence of most prevalent tumors in mice exposed for 60–61 weeks to 1,3-butadiene (20).

Tumor type and site	Sex	Airborne concentration, ppm ^a		
		0	625	1250
Malignant lymphoma	M	0/50	23/49	29/48
	F	1/50	10/49	10/49
Cardiac hemangiosarcoma	M	0/50	16/49	7/48
	F	0/50	11/48	18/49
Alveolar/bronchiolar adenoma/carcinoma	M	2/50	14/49	15/48
	F	3/49	12/48	23/49

^aNumber in denominator is number of animals examined histopathologically for the presence of the tumor of interest. All tumors listed were significantly increased in treated groups.

mammary gland were observed in those exposed to both low and high doses, and adenosquamous carcinomas were observed in low-dose females. The incidence of granulosa cell tumors of the ovary were also increased in dosed females. Each of these types of tumors occurred in mice that also had malignant lymphoma, cardiac hemangiosarcoma, or alveolar/bronchiolar adenoma/carcinomas. On the basis of these results the NTP concluded that there was clear evidence of carcinogenicity for 1,3-butadiene in male and female B6C3F₁ mice.

The largest difference observed between control mice and mice exposed to 1,3-butadiene in the NTP study is the incidence of malignant lymphomas among males. There is considerable uncertainty that the same response would occur in the general population of humans with exposure to 1,3-butadiene. This is largely because there is substantial evidence that the presence of an endogenous murine type C retrovirus (MuLV) in the B6C3F₁ strain mouse renders it uniquely sensitive to the development of lymphoma.

Murine leukemia viruses (MuLVs) were the first oncogenic mammalian viruses to be described more than 30 years ago (21). The predominant MuLV-induced response is lymphatic leukemia, which arises spontaneously in many mouse strains carrying MuLV. The type of leukemia induced is most often a thymic lymphoma composed of relatively immature T-cells. This type of lymphoma is particularly noteworthy since it is associated with 1,3-butadiene exposure in B6C3F₁ mice. It is also the type of lymphoma that increases when MuLV-carrying mice that normally have a very low incidence of spontaneous leukemia are exposed to other chemicals or irradiation (21). Thus, there is substantial evidence that the presence of MuLVs is an important factor in the etiology of lymphoma in B6C3F₁ mice exposed to 1,3-butadiene.

In attempting to clarify the role of MuLV in cancer in mice exposed to 1,3-butadiene, Irons and co-workers (22) exposed B6C3F₁ mice and NIH Swiss mice, which do not contain the endogenous virus, to 1250 ppm 1,3-butadiene. They noted a 60% incidence of lymphomas in the B6C3F₁ mice after 1 year (similar to that seen in the NTP study), but only 14% in the NIH Swiss mice (22). This suggests that the presence of the retrovirus modified quantitatively, but not qualitatively, the response to 1,3-butadiene. The possibility that the difference in response may be due to differences in pharmacokinetics,

or DNA repair capability, however, must also be considered.

These researchers also noted that the lesions induced in B6C3F₁ mice were likely to be of T-cell origin (similar to the radiation-induced and spontaneous lymphomas known to be associated with MuLV). Moreover, they have found that these lymphoma cells demonstrated elevated amounts of MuLV envelope antigens. When these lymphoma cells were cloned, an increased expression of the *c-myc* proto-oncogene was observed, suggesting that altered regulation of this particular gene, caused by the presence of the retrovirus, may be instrumental in the induction of lymphoma in mice exposed to 1,3-butadiene (23).

It thus appears that the presence of MuLV in B6C3F₁ mice enhances the incidence of malignant lymphoma in response to 1,3-butadiene exposure. Since humans are not known to carry this particular virus, extrapolation from these specific results to humans is extremely uncertain. Although human retroviruses do exist (HTLV 1–4), they are found in only a small proportion of the population, and it is not known if their presence in humans would affect susceptibility to lymphoma (or other neoplasm) in the same way as the murine retrovirus appears to affect development of lymphoma in mice exposed to 1,3-butadiene. When using data from this study for a risk assessment, therefore, a much stronger case can be made for relying on the incidence of tumors other than lymphoma (which presumably develop independently of the MuLV and solely as a result of exposure to 1,3-butadiene) in making interspecies extrapolations between mice and humans.

As noted in the section on metabolism and pharmacokinetics of 1,3-butadiene, there is some evidence that the metabolism of 1,3-butadiene in mice occurs at a faster rate and results in a greater amount of toxic metabolites than it does in rats (24,25). There is also limited evidence suggesting the rate and extent of metabolism of 1,3-butadiene to a putative toxic metabolite 3,4-epoxybutene in humans is more similar to that of rats than mice, although this remains to be more rigorously demonstrated (25). In any case, it is clear that there are interspecies differences in the rate and degree of metabolism of 1,3-butadiene that most likely contribute to the interspecies differences in susceptibility to its carcinogenic effect.

Until the pharmacokinetics of 1,3-butadiene in hu-

mans, rats, and mice are more thoroughly understood, it can be recognized only that interspecies differences in metabolism may contribute to interspecies differences in susceptibility to 1,3-butadiene induced cancer. Also mice, specifically, may be more susceptible than other species, including humans, because of their enhanced metabolism. Uncertainties regarding specific species differences in metabolism of 1,3-butadiene will contribute substantial uncertainty to the extrapolation of experimental results in either mice or rats to humans and to a quantitative risk assessment in general.

Hazleton Laboratories Europe Ltd. Rat Bioassay

In this study, sponsored by the International Institute of Synthetic Rubber Producers, Inc. (IISRP), Sprague-Dawley rats were exposed to air containing 0, 1000, or 8000 ppm 1,3-butadiene 6 hr/day, 5 days/week (26). The highest dose level used was limited by the explosive nature of higher concentrations of 1,3-butadiene. Exposures continued for 111 weeks for males and 105 weeks for females. Among females, significantly increased incidences of benign mammary tumors, thyroid follicular adenomas/carcinomas and uterine/cervical stromal sarcomas, and an almost significant ($p = 0.055$) increase in Zymbal gland tumors occurred in exposed animals, as illustrated in Table 2. Among males, significantly increased incidences of Leydig cell adenomas/carcinomas and pancreatic exocrine adenomas/adenocarcinomas occurred in exposed animals, also as illustrated in Table 2. In addition, there was a slight nonsignificant increase in

Zymbal gland adenomas/carcinomas in the males.

It should be noted that the results summarized in Table 2 are substantially different from the EPA's results that were used in the derivation of a unit cancer risk (UCR) for 1,3-butadiene based on these data (2). The results in Table 2 were derived from review of the entire final report of the 1,3-butadiene carcinogenicity bioassay in rats that was conducted by Hazleton Laboratories Europe Ltd. The reasons for the discrepancies between the results shown in Table 2 and those reported by EPA is unknown. In our review of the conduct of the study, as represented in the final report, we could find no reason to exclude these results from consideration, which was suggested by the EPA (2).

It should be kept in mind, however, that there are still considerable uncertainties involved in assuming that the response of rats to 1,3-butadiene is predictive of the human response.

Based on the results of the Hazleton rat bioassay, it is clear that inhalation of 1,3-butadiene is carcinogenic in rats. As discussed earlier, there is also evidence suggesting that 1,3-butadiene itself is not carcinogenic but that its metabolites, which are reactive epoxides, are. Therefore, there is the strong likelihood that carcinogenicity is dependent on the rate and degree to which 1,3-butadiene is metabolized. Only one study has compared the human metabolism of 1,3-butadiene with rats and mice (25). In that study, liver homogenates from a single human sample were shown to generate the same amount of epoxybutene as did rat liver homogenates and substantially less than that generated by mouse liver homogenates.

Table 2. Tumors in rats following inhalation exposure to 1,3-butadiene (26).

Tumor type and site	Exposure level, ppm		
	0	1000	8000
Females			
Numbers of rats with			
Mammary fibroadenoma*	40	75	67
Thyroid follicular adenoma/carcinoma*	0	4	11
Uterine/cervical stromal sarcoma*	1	5	7
Zymbal gland squamous carcinoma†	0	0	4
Total number of tumor-bearing animals having any of the tumors above except mammary fibroadenomas ^a	1/94	8/95	21/92
Total number of tumor-bearing animals having any of the significantly increased tumors above including mammary fibroadenomas ^b	41/99	77/97	72/96
Males			
Numbers of rats with			
Leydig cell adenoma/carcinoma*	0	3	8
Pancreatic exocrine adenoma/adenocarcinoma*	3	1	11
Zymbal gland adenoma/carcinoma	1	1	2
Total number of tumor-bearing animals having any of the above tumors ^c	4/96	4/96 ^d	20/87 ^d

*Significant differences between control and high exposure group by Fisher's exact test.

†Nearly significant difference ($p = 0.055$).

^aNumber in denominator is number of survivors at time of first tumor; uterine/cervical stromal sarcoma at 62 weeks.

^bNumber in denominator is number of survivors at time of first tumor; mammary fibroadenoma at 56 weeks.

^cNumber in denominator is number of survivors at time of first tumor; Zymbal gland adenoma at 66 weeks.

^dOne low-dose animal had both a pancreatic exocrine adenoma and a Leydig cell tumor, and one high-dose animal had both a Zymbal gland tumor and a Leydig cell tumor.

Genotoxicity of 1,3-Butadiene

Studies on the genotoxicity of 1,3-butadiene have indicated that 1,3-butadiene itself is not mutagenic in bacteria (27,28). Both of its putative metabolites, 3,4-epoxybutene and 1,2:3,4-diepoxymutane, however, are (29–32). In addition, 1,2:3,4-diepoxymutane has been shown to be mutagenic in fungi (33–35). Other evidence that these two metabolites are genotoxic include studies demonstrating the ability of 3,4-epoxybutene to alkylate DNA *in vitro* (36) and to induce sister chromatid exchanges (J. W. Allen, unpublished data). These studies also demonstrate that 1,2:3,4-diepoxymutane induces chromosomal aberrations and sister chromatid exchanges *in vivo* (37) and in cultured mammalian cells (38,39). 1,2:3,4-Diepoxymutane also increases the incidence of sex-linked recessive lethal mutations and broken chromosomes in *Drosophila* (40–42).

These experimental data are not directly applicable to a quantitative risk assessment. These results, however, are important in both the overall assessment of the carcinogenicity of a compound in the hazard identification step and in the interspecies and low-dose extrapolation of the dose-response evaluation step of risk assessment. For example, there is much current evidence that many known carcinogens are mutagens and that they can interact and cause damage to DNA. Thus, evidence that a chemical or its metabolites are mutagens and/or that they interact or cause damage to DNA is considered to be supportive evidence that it is a carcinogen. At the same time, evidence that a metabolite alone causes these effects suggests that metabolism is crucially important in the manifestation of any carcinogenic effect of the chemical and emphasizes the importance of considering metabolic similarities and differences in making interspecies extrapolations. Additionally, evidence that the metabolites of a compound are likely to be responsible for any carcinogenic effect emphasizes the desirability of basing a low-dose extrapolation of the dose-response curve on the effective doses of the toxic metabolite at the target site (which may be substantially different in different species) than on the administered dose of the parent compound.

Strength of the Evidence of Carcinogenicity

The scientific data described above attest to the carcinogenicity of 1,3-butadiene in rats and mice. They also demonstrate the strong likelihood that a metabolite (or metabolites) of 1,3-butadiene is probably responsible for its carcinogenicity. This is based on the fact that mice appear to be especially susceptible to the carcinogenic effect of 1,3-butadiene, that they also produce more metabolites at a faster rate than rats do, and that both the metabolites 3,4-epoxybutene and 1,2:3,4-diepoxymutane are directly mutagenic whereas 1,3-butadiene is not. This emphasizes the importance of interspecies differences in metabolism in assessing susceptibility to 1,3-butadiene carcinogenicity.

The epidemiological evidence is much less clear. The

data available when this analysis was originally conducted did not support a causal association between 1,3-butadiene exposure and increased mortality from any specific cancer type. More recent data are more supportive of a causal association, but inconsistencies between studies in types of neoplasms affected and a lack of evidence of a dose-response relationship render the epidemiologic data inconclusive. However, to compare the results obtained in the animal studies with the results of the epidemiologic studies, we have performed low-dose extrapolation of the experimental animal data.

Metabolism and Pharmacokinetics of 1,3-Butadiene

Although the inhalation of 1,3-butadiene induced cancer in both rats and mice, the mice showed a much greater incidence of tumors at substantially lower exposure levels than rats. This suggests either that mice were much more sensitive to the carcinogenic effects of 1,3-butadiene or that differences in one or more pharmacokinetic parameters (e.g., absorption, distribution, metabolism, elimination) existed between these species. The demonstration of such interspecies differences emphasizes the possibility that there may also be interspecies differences between rodents and humans in their response to 1,3-butadiene as a result of differences in pharmacokinetics and other biological factors.

Initial work on the metabolism of 1,3-butadiene was carried out by Malvoisin et al. (43) using rat liver microsomes. These authors incubated rat liver microsomes with 1,3-butadiene and found that it was metabolized to 3,4-epoxybutene (butadiene monoxide). They further demonstrated that induction of microsomal enzymes by pretreatment with phenobarbital enhanced conversion to 3,4-epoxybutene and that microsomal monooxygenase was the enzyme responsible. In a subsequent report these researchers compared the *in vitro* activity of the P-450 monooxygenase enzyme responsible for converting 1,3-butadiene to 3,4-epoxybutene, to the activity of the epoxide hydrolase enzyme, which they found reduced the epoxide to a putatively less toxic metabolite, 3-butene-1,2-diol (44). They found the monooxygenase to be about five times more active than the hydrolase. They, therefore, suggested that the putatively toxic butadiene monoxide would be formed more efficiently and rapidly than the putatively nontoxic butene diol.

In the most recent report in this series of investigations, Malvoisin and Roberfroid (45) demonstrated the *in vitro* conversion of 3,4-epoxybutene to both 1,2:3,4-diepoxymutane and 3,4-epoxy-1,2-butanediol in rat liver microsomes. Microsomal monooxygenase was responsible for both the conversion of 3-butene-1,2-diol, formed by the action of epoxide hydrolase on 3,4-epoxybutene, to 3,4-epoxy-1,2-butane diol and for the conversion of the 3,4-epoxybutene to 1,2:3,4-diepoxymutane. On the basis of the appearance of these metabolites in microsomes incubated with 3,4-epoxybutene and on their

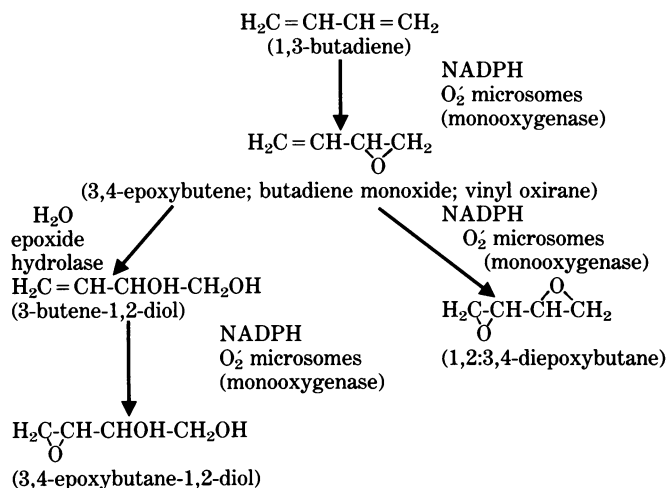


FIGURE 1. Proposed scheme for the metabolism of 1,3-butadiene based on Malvoisin and Roberfroid (46).

previous work, these authors proposed the scheme pictured in Figure 1 for the metabolism of 1,3-butadiene.

More recent studies (24,25,46-49) have confirmed this general pathway of metabolism and have demonstrated that the metabolism of 1,3-butadiene to epoxybutene was more rapid in mice than in rats, leading to higher tissue levels of the epoxide in mice than in rats. Schmidt and Loeser (25) also examined the ability of human liver and lung tissue homogenate (from a single individual) to metabolize 1,3-butadiene. They found that the rate of production of epoxybutene in liver was similar in the rat and human and lower than in mouse. Additionally, no epoxybutene was detected in human lung tissue homogenate exposed to 1,3-butadiene, while it was detected in rat lung homogenate and was 5- to 6-fold higher in mouse lung homogenate than in the rat. These differences may, at least in part, explain the differences in susceptibility among species.

Dose-Response Evaluation

This step in a risk assessment involves two types of extrapolation: interspecies extrapolation and low-dose extrapolation. The result of these processes is the derivation of a UCR that reflects the lifetime cancer risk to humans, given exposure to one unit of dose of the carcinogen. There is considerable uncertainty involved in each of these extrapolations because they are not ordinarily based on empirical data. The only data suitable for the dose-response evaluation of 1,3-butadiene are those from the two chronic animal bioassays. Even so, use of either of these data sets introduces uncertainty over and above that which is unavoidable when relying on experimental animal data to predict human responses. Extrapolation to humans and to low doses for each of these data sets are described below, along with a description of the uncertainties involved.

Mouse Study

Use of the data from the NTP mouse bioassay for risk assessment is complicated by several factors:

- As mentioned earlier, there is evidence that an endogenous murine retrovirus may be involved in the development of lymphoma in the 1,3-butadiene-exposed mice.
- There is evidence, particularly in males, of saturation of the carcinogenic response, since low-dose and high-dose animals showed similar tumor incidence. As a result, less information than usual is available regarding the shape of the dose-response relationship.
- The early termination of the study makes the standard low-dose extrapolation models, such as the multistage model, unsuitable.

To partially address some of these uncertainties, several procedures have been used:

- Separate extrapolations were conducted based on tumor-bearing animals having any of the tumors that showed a significant increase in incidence in one or both of the treated groups, and on the same animals except those that developed lymphoma.
- To better reflect the critical target-site dose, the retained doses of butadiene estimated based on studies performed for NTP and described by EPA (2) were used as shown in Table 3; a more detailed treatment of the pharmacokinetics of butadiene was not considered possible.
- To permit calculation of lifetime risk from the less-than-lifetime mouse study, the Hartley-Sielken general product model was used:

$$P(t,d) = 1 - \exp[-\alpha_0 + \alpha_1 d_1 + \dots + \alpha_k d^k] (\alpha_1 T + \alpha_m T^m)]$$

where $p(t,d)$ is the probability (risk) of developing a tumor from exposure to dose (d) by time (T), $\alpha_0, \alpha_1 \dots \alpha_k$ are dose-related parameters and $\beta_1 \dots \beta_m$ are time-related parameters estimated by fitting the experimental data on the time of identification of a tumor in an animal and its daily retained dose to the above equation.

The results of applying this model to the data from male mice (which predict the higher risk) are shown in Table 4 for an arbitrary lifetime daily dose of 1 mg/kg/day. Both maximum likelihood estimates and 95% upper confidence limits on the estimates are shown for two slightly different forms of the Hartley-Sielken model.

Rat Study

The Hazleton rat study (26) has fewer confounding factors associated with it. There is, therefore, likely to be less uncertainty associated with extrapolation to humans than would be the case with the NTP mouse study (20). One complicating factor in the use of the rat data is the fact that there is considerable evidence that the absorption of 1,3-butadiene in rats becomes saturated at levels above about 1000 ppm (24,49). This level of exposure therefore represents a level beyond which a

Table 3. Retention of 1,3-butadiene in mice exposed by inhalation [NTP data cited by EPA (2)].

Exposure		1,3-Butadiene and metabolites retained		Average daily dose 1,3-butadiene and metabolites, mg/kg/day ^a
ppm	μg/L	μmole/kg	mg/kg	
7	13	33	1.8	—
80	145	120	7.1	—
1040	1900	660	35.7	—
625	—	—	25.7 ^b	18.4
1250	—	—	38.9 ^c	27.8

^aAmount retained multiplied by 5 days exposure/7 days per week since mice were exposed for only 5 days/week in the NTP study.

^bEstimated by interpolation; corresponds to low dose in NTP bioassay.

^cEstimated by extrapolation; corresponds to high dose in NTP bioassay.

Table 4. Maximum likelihood estimates and 95% upper-confidence limits on extra-lifetime risk—based on tumors observed in male mice exposed to 1,3-butadiene via inhalation (20).

Estimates derived using Hartley-Sielken general product model where $P(T,d) = 1 - \exp[-(\alpha_0 + \alpha_1 d_1 + \dots + \alpha_k d^k)(\beta_1 T + \dots + \beta_m T^m)]$		
Tumors modeled	Estimate of extra risk, mg/kg/day ⁻¹	95% Upper-confidence limit on extra risk, mg/kg/day ⁻¹
k = 2, m = 2		
All significantly increased tumors ^a	1.8×10^{-1}	2.1×10^{-1}
All significantly increased tumors ^a except malignant lymphomas	9.5×10^{-2}	1.1×10^{-1}
k = 2, m = 3		
All significantly increased tumors ^a except malignant lymphomas	1.9×10^{-1}	2.2×10^{-1}

^aSignificantly increased tumors: malignant lymphoma, cardiac hemangiosarcoma, lung adenoma/carcinoma, forestomach papilloma/carcinoma.

Table 5. Retention of 1,3-butadiene in rats exposed by inhalation [NTP data cited by EPA (2)].

Exposure		1,3-Butadiene and metabolites retained		Average daily dose 1,3-butadiene and metabolites, mg/kg/day ^a
ppm	μg/L	μmole/kg	mg/kg	
70	125	40	2.2	—
930	1700	160	8.7	—
7100	12800	660	39.0	—
1000	—	—	10.5 ^b	7.4
8000	—	—	38.5 ^c	27.5

^aAmount retained multiplied by 5 days exposure/7 days per week since mice were exposed for only 5 days/week in the NTP study.

^bEstimated by interpolation; corresponds to low dose in Hazleton study (26).

^cEstimated by extrapolation; corresponds to high dose in Hazleton study (26).

proportional increase in the amount of 1,3-butadiene absorbed would not be expected to occur. In other words, it is very highly likely that the administered dose in the Hazleton rat bioassay does not proportionally reflect either the internal or effective dose. Moreover, estimates of internal dose based on an administered dose of 8000 ppm as will be done for these data will be very uncertain. On the other hand, there is clear evidence of a dose-response relationship in the incidence of tumors in both male and female rats, as shown in Table 2. This suggests that with the higher administered dose more of the toxic metabolite(s) are reaching target sites than with the lower administered dose. This emphasizes again that the shape of the dose-response curve, based on administered doses for these data, is very uncertain.

To provide both a conservative estimate of possible risk and an indication of the range of uncertainty, low-dose extrapolation was performed using all significantly increased tumor types using the multistage model, the model normally used by EPA, and two other plausible models: the Weibull model (50) and the Mantel-Bryan probit model (51). As with the mouse study, data on retention of inhaled 1,3-butadiene from studies conducted by NTP and cited by EPA (2) were used in an attempt to improve the accuracy of the extrapolation. These data are shown in Table 5, along with estimates of the retained daily doses in the bioassay based on the empirical data. Use of these data (and the corresponding mouse data) for risk assessment involves the assumption that the amount of 1,3-butadiene retained at any ex-

Table 6. Maximum likelihood estimates of dose coefficients and maximum likelihood and 95% upper-confidence limits on extra-lifetime risks—based on tumors observed in rats exposed to 1,3-butadiene via inhalation (26).

Estimates derived using the multistage model (GLOBAL 82) where $P(d) = 1 - \exp(-q_0 - q_1 d_1 - \dots - q_k d_k^k)$				
Sex	Tumors modeled	Maximum likelihood estimates of dose coefficients	Maximum likelihood estimates of excess risk at dose of 1 mg/kg/day	95% Upper-confidence limit on excess risk at dose of 1 mg/kg/day
Male	Significantly increased tumors ^a	$q_0 2.33 \times 10^{-2}$ $q_1 1.66 \times 10^{-3}$ $q_2 2.78 \times 10^{-4}$	1.93×10^{-3}	1.10×10^{-2}
	Significantly increased tumors ^a plus Zymbal gland adenoma/carcinoma	$q_0 2.13 \times 10^{-2}$ $q_1 6.30 \times 10^{-4}$ $q_2 2.94 \times 10^{-4}$	9.24×10^{-4}	9.36×10^{-3}
Female	Significantly increased tumors ^b (not including mammary fibro- adenomas)	$q_0 1.14 \times 10^{-2}$ $q_1 9.27 \times 10^{-3}$ $q_2 0$	9.27×10^{-3}	1.28×10^{-2}
	Significantly increased tumors ^c (including mammary fibroadenomas)	$q_0 7.21 \times 10^{-1}$ $q_1 3.43 \times 10^{-2}$	3.43×10^{-2}	4.97×10^{-2}

^aSignificantly increased tumors: Leydig cell adenoma/carcinoma, pancreatic exocrine adenoma/carcinoma.

^bSignificantly increased tumors: thyroid follicular adenoma/carcinoma, uterine/cervical stromal sarcoma, Zymbal gland squamous carcinoma, mammary fibroadenoma.

^cPoor goodness of fit of all three dose levels to multistage model: therefore, only control and low-dose groups modeled.

Table 7. Weibull and Mantel-Bryan model parameters based on tumors observed in rats exposed to 1,3-butadiene via inhalation (26).

Estimates derived using the Weibull model where: $P(d) = 1 - \exp(-a - bd^m)$				
Sex	Tumors modeled	Maximum likelihood estimates of dose coefficients	Maximum likelihood estimates of excess risk at dose of 1 mg/kg/day	95% Upper-confidence limit on excess risk at dose of 1 mg/kg/day
Male	Significantly increased tumors ^a plus Zymbal gland adenoma/carcinoma	$a 2.11 \times 10^{-2}$ $b 5.43 \times 10^{-4}$ $m 1.84$	5.43×10^{-4}	9.48×10^{-3}
Female	Significantly increased tumors ^b (not including mammary fibro- adenomas)	$a 1.15 \times 10^{-2}$ $b 9.29 \times 10^{-3}$ $m 1.00$	9.25×10^{-3}	1.27×10^{-2}

Estimates derived using the Mantel-Bryan model where: $P(d) = P_0 + (1 - P_0) \Phi(a + \log_{10} d)$, Φ = standard normal distribution function				
Male	Significantly increased tumors ^a plus Zymbal gland adenoma/carcinoma	$P_0 1.64 \times 10^{-2}$ $a -2.38$	8.6×10^{-3}	1.5×10^{-2}
Female	Significantly increased tumors ^b (not including mammary fibro- adenomas)	$P_0 1.01 \times 10^{-2}$ $a -2.25$	1.22×10^{-2}	2.0×10^{-2}

^aSignificantly increased tumors: Leydig cell adenoma/carcinoma, pancreatic exocrine adenoma/carcinoma.

^bSignificantly increased tumors: thyroid follicular adenoma/carcinoma, uterine/cervical stromal sarcoma, Zymbal gland squamous carcinoma, mammary fibroadenoma.

posure concentration is not affected by repeated exposure. The validity of this assumption is unknown.

The results of the low-dose extrapolation of the rat data are shown in Tables 6 and 7 for the various data sets and models used.

Exposure Assessment

As noted previously, measurements of workplace 1,3-butadiene concentrations in styrene rubber facilities provide the best available estimates for levels of human exposure. Workplace concentrations have generally not exceeded 10 to 20 ppm (2). Therefore, human risk to 1,3-butadiene is estimated, assuming 10 ppm. Also, in order to provide a range of possible risks, these are estimated, assuming 1 and 100 ppm exposure. Since these exposures are likely to be only occupational, it is assumed that exposure will be limited to 8 hr/day, 5 days/week, 50 weeks/year for 40 years over a lifetime of 70 years. An exposure to 1 ppm under this scenario is actually equivalent to a lifetime average daily dose of 0.062 mg/kg/day (as illustrated below), assuming 50% absorption of inhaled 1,3-butadiene.

1 ppm 1,3-butadiene = (2.2 mg 1,3-butadiene) (10 m³ air breathed/day) (5/7 days/week) (50/52 weeks/year) (40/70 years/lifetime) / (70 kg body weight)
= 0.062 mg/kg/day, lifetime average daily dose.

Risk Characterization

The final step in the risk assessment process (risk characterization) involves the evaluation of all relevant data pertaining to the likelihood that the substance in question is a human carcinogen, the quantitative results of the low dose and interspecies extrapolations, and information on the estimated or actual human exposure to the carcinogen. These components of risk assessment have been described in the previous sections.

Risk Estimates Based on Animal Data

Data described demonstrate that 1,3-butadiene induces a variety of tumor types in mice and rats. Using the estimates of cancer potency and the exposure scenarios presented above, the lifetime risk to humans that may exist as a result of exposure to 1,3-butadiene has been estimated. Because several data sets, extrapolation models, and levels of human exposure have been modeled (1, 10, and 100 ppm), several estimates of risk have been derived. The estimates are summarized in Tables 8 and 9.

When applied to the female rat data, the Weibull model gives results almost identical to those of the multistage model, since in both cases the best-fit is a linear curve. The male rat data give a nonlinear fit with the Weibull model and predict maximum likelihood es-

Table 8. Estimates of extra-lifetime risk based on tumors observed in rats exposed to 1,3-butadiene.

Basis of risk estimate	Estimated human exposure ^a	Multistage	Weibull model	Mantel-Bryan model
Maximum likelihood estimates of extra-lifetime risk.				
Based on significantly increased tumors in female rats	1 ppm	5.75×10^{-4}	5.76×10^{-4}	2.77×10^{-4}
	10 ppm	5.75×10^{-3}	5.74×10^{-3}	7.07×10^{-3}
	100 ppm	5.75×10^{-2}	5.60×10^{-2}	7.30×10^{-2}
Based on significantly increased tumors in male rats	1 ppm	1.04×10^{-4}	3.27×10^{-6}	1.65×10^{-4}
	10 ppm	1.14×10^{-3}	2.25×10^{-4}	4.80×10^{-3}
	100 ppm	2.10×10^{-2}	1.54×10^{-2}	5.59×10^{-2}
95% Upper confidence limits on extra-lifetime risk				
Based on significantly increased tumors in female rats	1 ppm	7.94×10^{-4}	7.93×10^{-4}	5.63×10^{-4}
	10 ppm	7.94×10^{-3}	7.90×10^{-3}	1.20×10^{-2}
	100 ppm	7.94×10^{-2}	7.63×10^{-2}	1.04×10^{-1}
Based on significantly increased tumors in male rats	1 ppm	6.82×10^{-4}	5.88×10^{-4}	3.70×10^{-4}
	10 ppm	6.82×10^{-3}	5.86×10^{-3}	8.79×10^{-3}
	100 ppm	6.82×10^{-2}	5.71×10^{-2}	8.47×10^{-2}

^aOccupational exposure to 1 ppm is equivalent to an average lifetime daily dose of 0.062 mg/kg.

Table 9. Estimates of extra-lifetime risk based on tumors observed in male mice exposed to 1,3-butadiene.

Basis of risk estimate	Estimated human exposure ^a	Estimates of extra-lifetime risk
Maximum likelihood estimates of extra-lifetime risk		
Based on all significantly increased tumors in male mice except malignant lymphomas	1 ppm	4.65×10^{-3}
	10 ppm	4.56×10^{-2}
	100 ppm	3.73×10^{-1}
95% Upper confidence limits on extra-lifetime risk		
Based on all significantly increased tumors in male mice except malignant lymphomas	1 ppm	5.50×10^{-3}
	10 ppm	5.34×10^{-2}
	100 ppm	4.26×10^{-1}

^aOccupational exposure to 1 ppm is equivalent to an average yearly daily dose of 0.109 mg/kg.

timates of risk somewhat lower than the multistage model (1.4-fold lower at 100 ppm; 5-fold lower at 10 ppm; and 32-fold lower at 1 ppm). However, the upper 95% confidence limit on risk for the Weibull model, based on the male rat data, is within 84% of the corresponding multistage model value for all three exposure levels.

The Mantel-Bryan model predicts risks that are within a factor of three of those predicted by the multistage model at all three exposure levels, as shown in Table 8.

The risk estimates presented in Table 9, derived from data on male mice, are higher by 5-fold to 40-fold than those derived from the rat data. This difference may, in part, be due to the apparently higher rate of metabolism of 1,3-butadiene to its monoepoxide in mice than in rats (25).

Conflict between Risks Predicted by Animal and Epidemiological Data

In contrast to the risk to humans predicted by use of either the mouse or rat data (as shown in Tables 8 and 9), there is, as discussed earlier, only equivocal evidence of an excess risk to workers who have been exposed to 1,3-butadiene.

The inconsistency of the predictions of risk made by EPA (2) based on the NTP mouse study has been demonstrated by IISRP (52), which calculated the extra deaths predicted by EPA's analysis of the NTP study (20), assuming different occupational exposure levels of 1,3-butadiene. IISRP compared those estimates to the number of deaths due to lymphopoietic cancer in the study by Matanoski et al. (10). This comparison is shown in Table 10 along with an estimate of the probability that the observed and predicted deaths are consistent.

It is clear that if average human exposure in the Matanoski study cohort was greater than 1 ppm (as it is likely to have been), the EPA estimates based on the NTP mouse study are incompatible with the observed response.

Similar calculations can be performed using the risk estimates derived from the Hazleton rat data, though the relevance is less clear because no excess of lymphopoietic cancers was seen in the rats (26). Using IISRP's estimates of average length of exposure (10 years) and average length of follow-up (18 years) in the Matanoski et al. study (10), the following calculations are possible:

At an exposure level of 1 ppm, working lifetime average exposure =

$$1 \times \frac{10 \text{ years}}{50 \text{ years}} \times \frac{240 \text{ days}}{365 \text{ days}} \times \frac{8 \text{ hr}}{24 \text{ hr}} = 0.044 \text{ ppm}$$

Individual lifetime risk =

$$0.044 \text{ ppm} \times 4.2 \times 10^{-3} (\text{ppm})^{-1} = 1.84 \times 10^{-4}$$

where 4.2×10^{-3} is the average cancer potency for male and female rats from Table 6.

Expected excess cases =

$$1.84 \times 10^{-4} \times 13920 \times 18/50 = 0.923$$

Similarly, the expected excess cases for 5, 10, and 25 ppm exposure levels can be calculated as 4.6, 9.2, and 23.1, respectively.

Table 11 shows an analysis similar to that in Table 10, but it is based on our assessment of the rat data. Because the rat data predict lower risks, these data do not overpredict as much as the mouse data do, though they still overpredict somewhat. This improvement may partly be attributed to the apparent closer similarity between the rat and human than between the mouse and human in the pharmacokinetics of 1,3-butadiene.

As noted earlier, the recent epidemiology study by Downs et al. (11) did show a slight excess of lymphopoietic cancer. However, this excess is significantly less than would be predicted from the results of the NTP

Table 10. Excess lymphopoietic cancer deaths predicted among study cohort of Matanoski et al.^a by EPA modeling of NTP mouse data (52).^a

Assumed exposure level, ppm ^b	Extra deaths predicted	Total deaths predicted	Probability of observing 40 deaths given EPA's predictions
1	5.5	52.6	0.09
5	27.5	74.6	1.8×10^{-5}
10	54.9	102.0	6.4×10^{-12}
25	137.3	184.4	4.1×10^{-36}

^aAssumes all excess risk to humans is due to lymphopoietic cancer.

^bBecause no good estimates of exposure level exist, the effect of assuming different levels is examined.

Table 11. Excess lymphopoietic cancer deaths predicted among study cohort of Matanoski et al. (10) by EPA modeling Hazleton rat data (26).

Assumed exposure level, ppm	Extra deaths predicted	Total deaths predicted	Probability of observing 40 deaths given EPA's predictions
1	0.92	48.0	0.138
5	4.6	51.7	0.055
10	9.2	56.3	0.014
25	23.1	70.1	6.7×10^{-5}

mouse study, assuming worker exposure levels were in the range of 10 to 20 ppm (or higher). The excess in the Downs study is, however, numerically consistent with the excess total risk predicted on the basis of the Hazleton rat bioassay.

The apparent consistency between the Downs et al. study (11) and the rat data must be tempered by the fact that the sites where an excess of tumors occurred in the rat study (Leydig cells, pancreas, thyroid, uterus/cervix, and possibly Zymbal gland) are not related to the sites apparently affected in the Downs et al. study (lymphohematopoietic cancer). As such, the apparent numerical consistency is likely coincidental, particularly since no such increase in lymphohematopoietic cancer was observed in the larger, statistically more powerful study by Matanoski et al. (10,12).

We have not had the opportunity to examine how the predictions of the animal data match the recently updated epidemiologic data; such an evaluation would be informative.

Conclusions

The available epidemiologic data provide only equivocal evidence of a carcinogenic risk from human exposure to 1,3-butadiene. Inconsistencies between the predictions of risk on the basis of the animal studies and human experience call for caution in attempting to quantify human risk on the basis of the animal data. These inconsistencies may arise because of the probable involvement of murine leukemia virus in mice and because the target organs that are affected in the rats show no sign of elevated cancer incidence in humans. Also the possible targets in humans are not affected in rats. It is to be hoped that both the continued follow-up of the occupationally exposed cohorts and further research on 1,3-butadiene pharmacokinetics and its possible mechanism of action will improve our understanding of possible human risk from the gas.

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